

Restriction Digestion

1. Thaw the plasmids or BioBricks to be digested.
2. Give a quick spin to the enzymes.
3. Place the reagents needed on ice.
4. Add 500ng of DNA to a PCR tube.
5. Add 1 μ L of each enzyme. Restriction enzymes used for 3A assembly are the followings:
 - Upstream Part: EcoRI-HF and SpeI
 - Downstream Part: XbaI and PstI
 - Destination Plasmid: EcoRI-HF and PstI
6. Add 5 μ L of 10X NEBuffer 2.1.
7. Bring the volume up to 50 μ L with nuclease free water.
8. Incubate the restriction digest at 37°C for 3 hours and then heat inactivate at 80°C for 20 minutes.